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Letter

Attenuated response to SARS-CoV-2 vaccine in patients with asymptomatic precursor stages of multiple myeloma and Waldenstrom macroglobulinemia

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Patients with hematologic malignancies, including multiple myeloma (MM) and Waldenstrom macroglobulinemia (WM), experience worse outcomes in response to SARS-CoV-2 infection and exhibit suboptimal responses to vaccination due to humoral and cellular immunity defects and immunosuppressive therapy (Aleman et al., 2021; Greenberger et al., 2021; Griffiths and Segal, 2021). Multiple myeloma (MM) is the second most common hematologic malignancy in the United States and is always preceded by monoclonal gammopathy of undetermined significance (MGUS) and smoldering myeloma (SMM), two precursor conditions that affect approximately 3%–5% of the population over 50 years of age, with African Americans carrying three times the risk (Marinac et al., 2020). More than 10 million individuals in the United States are estimated to have MGUS, and we have previously shown that MGUS and SMM exhibit immune dysregulation (Zavidij et al., 2020). Therefore, we reason that patients with precursor plasma cell dyscrasias may also be at risk for SARS-CoV-2 infection and suboptimal response to vaccination.

We launched the IMmune Profiling with Antibody-based COVID-19 Testing (IMPACT) national cohort study in November 2020 to characterize how the short- and long-term effects of SARS-CoV-2 vaccination are modified by underlying immune dysregulation due to precursor plasma cell dyscrasias. The IMPACT study is a prospective study at Dana-Farber Cancer Institute (DFCI) that enrolled participants from three prospectively followed cohorts: the PCROWD study (NCT02269592), the PROMISE study (NCT03689595), and the CureCloud (NCT03657251) collaborative study with the Multiple Myeloma Research Foundation (MMRF). A questionnaire regarding prior SARS-CoV-2 infection or vaccination was sent to all participants.

Between November 2020 and October 2021, 3,005 individuals completed a questionnaire assessing prior SARS-CoV-2 infection or vaccination (vaccine type and dates of administration). Self-reported data were collected on demographic variables (age, sex, race), diagnosis, past medical history of malignancies, and family history of malignancies. Chart review was conducted to retrieve patient clinical variables, including diagnosis, prior therapeutic interventions, and clinical laboratory test results, including monoclonal protein (M-spike) free light-chain (FLC) ratio, albumin, creatinine, hemoglobin, and bone marrow (BM) plasma cell infiltration percentage. A detailed description of the participants who answered the questionnaire is presented in Table S1A.

Most individuals in our cohort received a full vaccination course (2,771, 92%) (two doses of BNT162b2 or mRNA-1273 or one dose of Ad26.COV2.S), including 269 individuals (8.9%) who received a third dose, while 234 individuals (7.8%) remained unvaccinated. 1,385 (46%) and 1,090 (36%) participants received mRNA vaccines (BNT162b2 or mRNA-1273, respectively), and 145 (4.8%) participants received an adenovirus-vector vaccine (Ad26.COV2.S). SARS-CoV-2 infection was observed in 253 (8.4%) individuals, including 33 (1.1%) individuals who experienced a breakthrough infection after a full vaccination course. Indeed, out of all 974 patients with precursor diseases, 15 (1.5%) patients experienced a breakthrough infection.

To evaluate the humoral immune response, we employed one clinically validated and two research-level SARS-CoV-2 spike protein-binding IgG antibody tests. We used a clinical laboratory improvement amendment (CLIA)-certified antibody test with results returned to patients, including a qualitative test (Quest Diagnostics code #39504), and beginning in March 2021, a semiquantitative test (Quest Diagnostics #34499). On the research level, we used enzyme-linked immunosorbent assays (ELISA) and time-resolved Förster resonance energy transfer (TR-FRET) tests (Supplemental information).

Results for all three tests were available on 261 samples. We compared the results of each assay to the CLIA-certified semi-quantitative test within its quantitative range (1–20 index) (n = 22 samples). The

ELISA results (r = 0.737, p < 0.001), but not the TR-FRET results (r = 0.159, p = 0.481), were significantly correlated with the clinical test results (Figure S1A). Since the TR-FRET assay does not include washing steps while relying on anti-human IgG antibodies to detect anti-SARS-CoV-2 spike protein IgG antibodies, we hypothesized that the increased IgG immunoglobulin levels inherent in plasma cell dyscrasias could interfere with this assay. Further analysis using samples spiked with various concentrations of positive control antibody confirmed that immunoglobulin interference occurred in the TR-FRET assay (Figure S1B). Accordingly, we used antibody titers measured by ELISA for the analysis. We urge providers to be aware of the potential immunoglobulin interferences in immunoassays especially when applied to patients with plasma cell dyscrasias.

We analyzed 1,350 plasma samples from 628 individuals who had received a vaccination, including 201 (32%) individuals with MGUS, 221 (35%) with SMM, 40 (6.4%) with smoldering WM (SWM), 66 (10%) with MM, and 100 (16%) healthy controls (Table S1B). Among them, 547 (87%) individuals submitted at least one blood sample after full vaccination, and 209 (33%) patients submitted multiple samples after a full vaccination course, with a median of 2 (range, 2–6) samples per patient. Patients with SMM were stratified by the 2/20/20 progression risk criteria into low-risk, intermediate-risk, and high-risk groups (Lakshman et al., 2018). While the standard of care for patients with SMM is active monitoring until progression to overt MM, our cohort included 41 (6.5%) SMM patients who have received therapies and 17 (0.3%) SMM patients who were actively treated at the time of blood collection (Table S1C).

To determine factors that contributed to antibody responses to SARS-CoV-2 vaccination, we fit a linear model on antibody titers (Figure S1C and Table S1D). Consistent with previous reports (Bird et al., 2021; Greenberger et al., 2021; Stampfer et al., 2021; Van Oekelen et al., 2021), patients diagnosed with MM were significantly more likely to show attenuated humoral immune response (β −0.44, 95% CI: −0.67, −0.21, p < 0.001). Importantly, patients with asymptomatic SMM had significantly attenuated humoral immune response regardless of their 2/20/20 risk stage, even with low-risk SMM (low-risk: β: −0.22, 95% CI: −0.42, −0.03, p = 0.027; intermediate-risk: β: −0.40, 95% CI: −0.61, −0.19, p < 0.001; and high-risk: β: −0.53, 95% CI: −0.88, −0.18, p = 0.003). A diagnosis of MGUS (β −0.13, 95% CI: −0.28, 0.03, p = 0.103) or SWM (β: −0.15, 95% CI: −0.36, 0.07, p = 0.181) was not significantly associated with attenuated antibody response. However, the coefficients were negative, and we may be underpowered to detect a significant difference for this effect size. Therefore, this result should be interpreted with caution. In addition to disease state, male sex (β: −0.12, 95% CI: −0.22, −0.02, p < 0.010), elapsed time after vaccination (β: −0.00, 95% CI: −0.01, −0.00, p < 0.001), and receiving the BNT162b2 vaccine (β: −0.38, 95% CI: −0.48, −0.29, p < 0.001) were also associated with attenuated antibody response, while SARS-CoV-2 infection prior to vaccination was associated with enhanced antibody response (β: 0.78, 95% CI: 0.58, 0.98, p < 0.001). Collectively, our results indicate that the humoral immune response is attenuated in asymptomatic SMM patients, even those with low-risk SMM and low tumor burden. As we do not screen for SMM, these individuals are largely undiagnosed and would not know that they may be at higher risk for SARS-CoV-2 infection.

Patients with SMM are a heterogeneous population, encompassing patients with indolent MGUS-like disease and patients who will progress to overt MM within 5 years of diagnosis. To determine whether all SMM patients are equally at risk for attenuated humoral immune response, we fit a linear model within the sub-cohort of SMM patients adjusting for clinical variables that are commonly used to monitor the risk of progression in patients with SMM (Figure S1D and Table S1E). We observed that a higher percentage of BM plasma cell infiltration (β: −0.20, 95% CI: −0.37, −0.03, p = 0.018) and a higher FLC ratio (involved/uninvolved light chain, β: −0.16, 95% CI: −0.32, 0.01, p = 0.060), both markers of advanced disease, were associated with lower antibody titers post-vaccination. Prior SARS-CoV-2 infection (β: 0.92, 95% CI: 0.57, 1.27, p < 0.001), receiving the BNT162b2 (β: −0.38, 95% CI: −0.58, −0.18, p < 0.001), and longer elapsed time (β: −0.01, 95% CI: −0.01, −0.00, p < 0.001) after vaccination were again significantly associated with lower antibody response. These results indicate that the more advanced the SMM tumor is, the worse the patient's humoral immune response to SARS-CoV-2 vaccination will be, which may help inform future vaccination strategies in these patients.

While patients with hematologic malignancies are encouraged to receive a third dose of vaccination, we do not have evidence that a third dose may indeed overcome disease-associated immune dysregulation. Therefore, we examined the effect of a third dose of mRNA vaccination on antibody titers in 25 patients (6 MGUS, 10 SMM, 2 SWM, and 7 MM) who received three vaccine doses and submitted blood samples both after the second dose and after the third dose. In these patients, we observed a significant increase in antibody titer after receiving the third dose (paired t test, p = 0.002) (Figure S1E). To determine whether these higher titers could be considered acceptable, we compared patient antibody titers post-third dose (13 MGUS, 12 SMM, 2 SWM, and 31 MM) to those of healthy individuals after the second dose. Since all available samples after the third dose were collected within 65 days post-vaccination (median 33; range, 1–65), we restricted this comparison to samples of healthy individuals collected within 65 days of the second dose of vaccine (median 41; range, 2–64). We observed highly variable antibody titers after the third dose in patients, but, overall, they were comparable to titers post-second dose in healthy individuals (p = 0.833) (Figure S1F). While we do not know how antibody titers post-third dose in patients with plasma cell dyscrasias are compared to titers post-third dose in healthy individuals, our results indicate that this patient population may require one dose more than healthy individuals to reach similar antibody levels. With longer follow-up, we will be able to assess the dynamics of antibody titer waning over time in patients with precursor plasma cell dyscrasias compared to healthy individuals and determine whether the intervals between doses should perhaps be shorter for our patients.

Our model suggested that the humoral immune response in SMM patients with

prior treatment history within 2 years of vaccination was comparable to that of healthy individuals (β: 0.09, 95% CI: −0.09, 0.28, p = 0.311), even though having SMM was a significant predictor of attenuated response (Figure S1C). Indeed, SMM patients with prior treatment history had significantly lower tumor burden than untreated SMM patients in terms of M-spike level ($p < 0.001$) and BM plasma cell infiltration percentage ($p < 0.001$). While early therapy is still under investigation as a strategy in patients with high-risk SMM, these encouraging results suggest that the earlier therapeutic interventions in high-risk SMM patients may effectively downstage SMM patients who may have an improved antibody response to vaccination. Benefits from long-term immunomodulation due to therapy are also possible, but these data need to be evaluated further. In contrast, receiving active treatment for SMM while being vaccinated was near significant as a predictor of attenuated antibody response (β: −0.21, 95% CI: −0.44, 0.02, p = 0.078), consistent with prior reports observed in symptomatic MM (Stampfer et al., 2021; Van Oekelen et al., 2021) (Figure S1C).

Finally, we identified patients who experienced a breakthrough infection after a full vaccination course. Among them, we obtained blood samples from seven patients (2 MGUS, 5 SMM). Their antibody titer after full vaccination, but before infection, (median 1.78, range 1.30–1.83) was comparable to that in healthy individuals after vaccination (p = 0.691). This indicates that factors beyond humoral immune response may contribute to breakthrough infections. We are investigating whether the cellular immune response should also be considered to fully define vaccine-induced immune responses in patients with plasma cell dyscrasias.

In conclusion, our study demonstrates that the humoral immune response to SARS-CoV-2 vaccination is suboptimal, not only in patients with MM and other cancer patients receiving therapy but also in precursor asymptomatic patients, including low-risk SMM. Since early stages of hematologic malignancies were not screened routinely, many individuals who are not currently diagnosed with these precursor conditions may be at risk for an attenuated response to SARS-CoV-2 vaccination and may not be aware of their risk. The third vaccine dose improved their attenuated humoral immune response. Future studies examining whether breakthrough infections are indeed associated with other precursor conditions need to be explored. Providers should be aware that a substantial subset of patients with plasma cell dyscrasias, even if asymptomatic, may be at high risk of breakthrough SARS-CoV-2 infections.

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.ccell.2021.12.003.

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DECLARATION OF INTERESTS

E.S.F. is a founder, equity holder, and scientific advisor for Civetta Therapeutics, Jengu Therapeutics (board of directors), and Neomorph Inc. and a consultant to EcoR1 Capital, Sanofi, Deerfield, and Avilar. The Fischer lab receives or has received research funding from Novartis, Astellas, Deerfield, Interline, and Ajax. I.M.G. is a consultant for AbbVie, Adaptive, Bristol Myers Squibb, Celgene Corporation, Cellectar, CohBar, Curio Science, Dava Oncology, Genetech, Huron Consulting, Karyopharm, Magenta Therapeutics, Menarini Silicon Biosystems, Oncopeptides, Pure Tech Health, Sognef, Takeda, and The Binding Site; an advisor for Mind Wrap Medical, LLC; and an advisor and consultant for Amgen, Aptitude Health, GlaxoSmithKline, GNS Healthcare, Janssen, Pfizer, and Sanofi. I.M.G.'s spouse, William Savage, MD, PhD, is CMO and equity holder of Disc Medicine.

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Supplemental information

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Supplemental Information

Table S1. Patient demographics and results of multiple variate analysis

(A) Patient demographics of 3,005 individuals enrolled in the IMPACT study. (B) Patient demographics of 628 individuals analyzed by ELISA. (C) Treatment history of 58 SMM patients. (D, E) Results of multivariate analysis using multiple linear regression models. Association between vaccine-induced antibody titer and clinical variables among patients with plasma cell dyscrasias (D) or patients with SMM (E) was examined. Data of healthy individuals or low-risk SMM patients were used as a control, respectively. Data represented as β coefficient +/- 95% confidence interval.

Table S1A: Characteristics of 3,005 individuals enrolled in this study

		Diagnosis					
	Total n=3,005 (%)	**Healthy n=1054 (%)**	**MGUS n=547 (%)**	**SMM n=346 (%)**	**SWM n=81 (%)**	**MM n=82 (%)**	**Others[#] n=895 (%)**
Age							
Median (range)	60 (18–93)	58 (25–78)	64 (22–93)	64 (30–83)	68 (51–87)	67 (30–86)	53 (18-80)
Gender							
Male	975 (32)	289 (27)	194 (35)	149 (43)	39 (48)	45 (55)	259 (29)
Female	1992 (66)	765 (73)	353 (65)	197 (57)	42 (52)	37 (45)	598 (67)
Not answered	38 (2)	-	-	-	-	-	38 (4)
Race							
White or Caucasian	2689 (90)	940 (89)	464 (85)	317 (92)	78 (96)	81 (99)	809 (91)
Black or African American	162 (5)	69 (7)	32 (6)	13 (4)	-	1 (1)	47 (5)
Others	154 (5)	45 (4)	51 (9)	16 (4)	3 (4)	-	39 (4)
Vaccine							
Received full vaccination	2771 (92)	988 (94)	499 (91)	328 (95)	78 (96)	80 (98)	798 (89)
BNT162b (Pfizer)	1385 (46)	496 (47)	239 (44)	173 (50)	39 (48)	42 (52)	396 (44)
mRNA-1273 (Moderna)	1090 (36)	429 (41)	200 (36)	119 (35)	33 (41)	31 (38)	278 (31)
Ad26.COV2.S (J&J)	145 (5)	57 (5)	22 (4)	11 (3)	1 (1)	6 (7)	48 (5)
Unknown	151 (5)	6 (1)	38 (7)	25 (7)	5 (6)	1 (1)	76 (9)
Never received vaccination	234 (8)	66 (6)	48 (9)	18 (5)	3 (4)	2 (2)	97 (11)
3rd dose vaccine							
Received 3rd dose vaccine	269 (9)	25 (2)	62 (11)	71 (21)	20 (25)	59 (72)	32 (3)
SARS-CoV-2 PCR positive							
Before full vaccination	176 (6)	56 (5)	36 (7)	18 (5)	3 (4)	3 (4)	60 (7)
After full vaccination	33 (1)	8 (1)	9 (2)	6 (2)	-	-	10 (1)
Never received vaccination	44 (1)	12 (1)	9 (2)	6 (2)	-	1 (1)	16 (2)

[#]There were 836 participants from the PROMISE study who had pending diagnostic studies and therefore, we had no information at the time of writing this manuscript on their status for precursor myeloma. In addition, PCROWD enrolled other hematological malignancies (n = 59) and these included the following cases that also participated in the vaccine questionnaire: myeloproliferative neoplasms n = 15, monoclonal B cell lymphocytosis n =8, early MDS n = 6, solitary plasmacytoma n =2, and 28 other cases of precursor hematological conditions including early-stage asymptomatic low-grade lymphoma and POEMS. Of note, those cases were not included in the plasma samples analyzed in the manuscript.

Table S1B: Characteristics of 628 individuals analyzed using ELISA

		Diagnosis				
	Total n=628 (%)	**Healthy n=100 (%)**	**MGUS n=201 (%)**	**SMM n=221 (%)**	**SWM n=40 (%)**	**MM n=66 (%)**
Age						
Median (range)	64 (30–90)	59 (41–74)	64 (34–90)	65 (39–83)	69 (51–87)	64 (30–86)
Gender						
Male	242 (39)	26 (26)	67 (33)	99 (45)	19 (48)	31 (47)
Female	386 (61)	74 (74)	134 (67)	122 (55)	21 (52)	35 (53)
Race						
White or Caucasian	584 (93)	89 (89)	182 (91)	209 (94)	39 (98)	65 (98)
Black or African American	25 (4)	7 (7)	14 (7)	4 (2)	-	-
Others	19 (3)	4 (4)	5 (2)	8 (4)	1 (2)	1 (2)
Vaccine Type						
BNT162b (Pfizer)	334 (53)	45 (45)	102 (51)	130 (59)	19 (48)	38 (58)
mRNA-1273 (Moderna)	262 (42)	47 (47)	88 (44)	84 (38)	19 (48)	24 (36)
Ad26.COV2.S (J&J)	32 (5)	8 (8)	11 (5)	7 (3)	2 (4)	4 (6)
SARS-CoV-2 PCR positive test						
Before full vaccination	34 (5)	7 (7)	12 (6)	12 (5)	3 (8)	-
After full vaccination	10 (2)	1 (1)	3 (1)	6 (3)	-	-
Blood collection						
Both pre-and post-vaccination	272 (43)	28 (28)	74 (37)	136 (62)	18 (45)	16 (24)
Only pre-vaccination	81 (13)	25 (25)	32 (16)	17 (7)	5 (12)	2 (3)
Only post-vaccination	275 (44)	47 (47)	95 (47)	68 (31)	17 (43)	48 (73)
3rd dose vaccine						
Received 3rd dose vaccine	124 (20)	3 (3)	28 (14)	38 (17)	10 (25)	45 (68)
Blood collection after 3rd dose	59 (9)	1 (1)	13 (6)	12 (5)	2 (5)	31 (47)

Table S1C. Treatment history in SMM patients

	Total n=58 (%)	**On therapy n=17 (%)**	**Post-treatment n=41 (%)**
Single-agent daratumumab	24 (41)	5 (29)	19 (46)
Daratumumab/Bortezomib/Lenalidomide/Dexamethasone	7 (12)	7 (41)	0 (0)
Daratumumab/Carfizomib/Lenalidomide/Dexamethasone	1 (2)	1 (6)	0 (0)
Ixazomib/Lenalidomide/Dexamethasone	25 (43)	4 (24)	21 (51)
Lenalidomide/Dexamethasone	1 (2)	0	1 (3)

Table S1D. Model of the association between antibody titer and clinical variables in patients with plasma cell dyscrasias

Predictors	**Coefficient**	**95% CI**	***p-values***
MGUS	-0.13	-0.28 – 0.03	0.103
Low-risk SMM	-0.22	-0.42 – -0.03	**0.027**
Intermediate-risk SMM	-0.40	-0.61 – -0.19	**<0.001**
High-risk SMM	-0.53	-0.88 – -0.18	**0.003**
SWM	-0.15	-0.36 – 0.07	0.182
MM	-0.44	-0.67 – -0.21	**<0.001**
Days after full vaccination	-0.00	-0.01 – -0.00	**<0.001**
Age	-0.04	-0.08 – 0.01	0.163
Male	-0.12	-0.22 – -0.02	**0.019**
Self-reported race White	-0.29	-0.58 – 0.01	0.056
SARS-CoV-2 infection prior to vaccination	0.78	0.58 – 0.98	**<0.001**
Receiving BNT162b2	-0.38	-0.48 - -0.29	**<0.001**
Treatment status (active treatment)	-0.21	-0.44 – 0.02	0.078
Treatment status (post-treatment)	0.09	-0.09 – 0.28	0.311

N = 628. CI = confidence interval. Coefficient, CI, and p-values from multiple regression model. α=0.05.

Table S1E. Model of the association between antibody titer and clinical variables in SMM patients			
Predictors	**Coefficient**	**95% CI**	***p-values***
Days after full vaccination	-0.01	-0.01 – -0.00	**<0.001**
Intermediate-risk based on 2/20/20	0.08	-0.21 – 0.37	0.583
High-risk based on 2/20/20	0.59	-0.04 – 1.22	0.065
Age	0.00	-0.09 – 0.10	0.944
Male	-0.20	-0.42 – 0.01	0.064
Self-reported race White	-0.35	-0.83 – 0.13	0.156
SARS-CoV-2 infection prior to vaccination	0.92	0.57 – 1.27	**<0.001**
Receiving BNT162b2	-0.38	-0.58 – -0.18	**<0.001**
Immunofixation (IgG subtype)	-0.17	-0.48 – 0.14	0.272
Hemoglobin (g/dL)	-0.00	-0.13 – 0.13	0.987
Albumin (g/dL)	0.02	-0.10 – 0.13	0.805
Creatinine (mg/dL)	0.03	-0.09 – 0.15	0.570
M spike protein (g/dL)	-0.11	-0.25 – 0.04	0.141
Kappa to lambda free light chain ration	-0.16	-0.32 – 0.01	0.060
Plasma cell percentage on BM biopsy (%)	-0.20	-0.37 – -0.03	**0.018**
Treatment status (active treatment)	-0.26	-0.54 – 0.02	0.068
Treatment status (post-treatment)	-0.02	-0.24 – 0.19	0.825

N = 193. CI = confidence interval. Coefficient, CI, and p-values from multiple regression model. α=0.05.

Supplemental figure 1

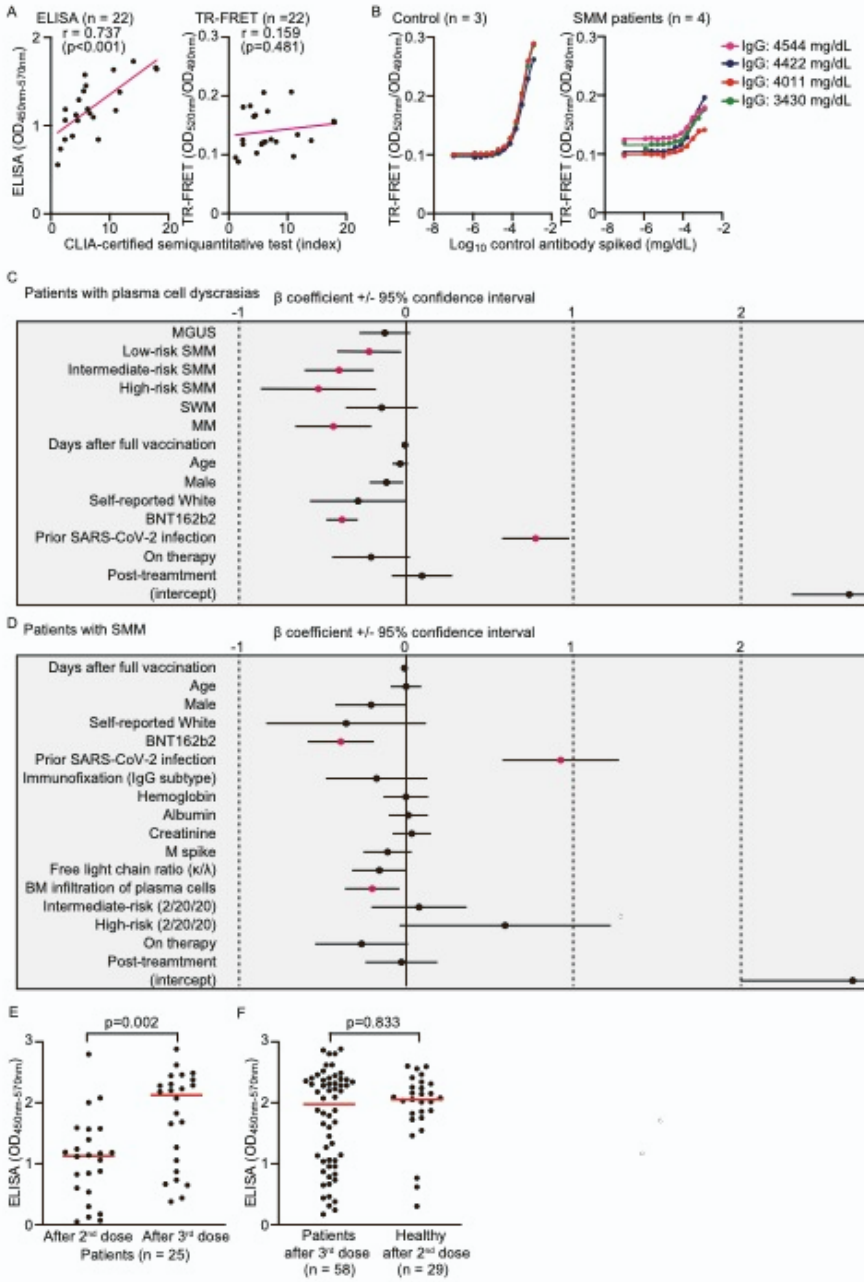

Figure S1. SARS-CoV-2 anti-spike protein (S) IgG antibody response in patients with a spectrum of plasma cell dyscrasia after full vaccination

(A) The correlation between clinical-grade and research-level antibody tests. Each dot indicates an individual test result (n = 22). The x-axis represents the CLIA-certified semiquantitative test results within its quantitative range (1 – 20 index) and Y-axis represents ELISA measurements ($OD_{450nm-570nm}$) (left) or TR-FRET measurements (OD_{520nm}/OD_{490nm}) (right), respectively. Pearson correlation was applied (r; correlation coefficient); $\alpha = 0.05$. Magenta line represents the regression line. (B) TR-FRET measurements using blood samples taken from individuals without plasma cell dyscrasias (left, n = 3) or SMM patients (right, n = 4) spiked with various concentrations of positive control antibody (CR3022 IgG). Each dot represents an individual test result, and each line represents the dose-response curves based on a non-linear variable slope model. The increase in TR-FRET measurements observed in control samples after adding the positive control antibody was masked in samples obtained from SMM patients. The IgG level (mg/dL) of each sample is labeled on the right. (C, D) Multivariate analysis of vaccine-induced antibody response among patients with plasma cell dyscrasias (C) or in patients with SMM (D). Data of healthy individuals or low-risk SMM patients was used as the control, respectively. A multiple linear regression model was applied. Data represented as β coefficient +/- 95% confidence interval. Magenta circles indicate statically significant results. All tests were two-sided, with $\alpha = 0.05$. (E) Anti-SARS-CoV-2 IgG antibody levels before and after receiving a 3rd vaccine dose in patients with plasma cell dyscrasia (n = 25; 6 MGUS, 10 SMM, 2 SWM, and 7 MM). P-value from Wilcoxon matched-pairs signed rank test is displayed; $\alpha = 0.05$. (E) Antibody titers in patients with plasma cell dyscrasias after the 3rd dose (n = 58; 13 MGUS, 12 SMM, 2 SWM, and 31 MM) and healthy individuals after the 2nd dose (n =29). P-value from Mann-Whitney test is displayed; $\alpha = 0.05$.

Supplemental methods

Study information and patient selection

The IMPACT study (Dana-Farber Cancer Institute IRB #20-332) is a prospective observational cohort study in collaboration with Multiple Myeloma Research Foundation (MMRF), which enrolled individuals nationwide who have been diagnosed with a spectrum of plasma cell dyscrasias and healthy individuals with risk factors for MM. These risk factors are age (40 – 75 years), self-identified Black or African American race, or having family relatives with hematologic malignancies. These participants were enrolled on other studies that allowed them to participate in the IMPACT study including the PROMISE study (a study to screen high-risk

individuals for myeloma specifically focusing on Black Americans, NCT03689595), the PCROWD study (an observational prospective cohort study of MGUS and SMM, NCT02269592), and the CureCloud study (an MMRF study recruiting SMM and active myeloma patients for direct-to-patient access of liquid biopsy genomic results, NCT03657251). A questionnaire regarding prior SARS-CoV-2 infection or vaccination (vaccine type and dates of administration) was sent out to all participants in IMPACT. All participants enrolled in one of these studies who answered the questionnaire were included in this study [913 IMPACT study (435 PROMISE, 396 PCROWD, and 82 CureCloud), 1558 PROMISE study, and 534 PCROWD]. Of 913 participants enrolled in IMPACT, 514 individuals received clinically approved SARS-CoV-2 IgG antibody test provided by Quest Diagnostics (Secaucus, NJ). All participants provided informed consent prior to the collection of data and specimens. Self-reported data were collected on demographic variables (age, sex, race), diagnosis, vaccine type and dates, prior SARS-CoV-2 infections confirmed by PCR test, past medical history of malignancies or autoimmune diseases, and family history of malignancies. Chart review was conducted to retrieve patient clinical variables when available (diagnosis, prior history of treatment, and clinical laboratory test results). The clinical laboratory test results closest to the date of 1st dose of vaccine were adapted. Individuals who received two doses of mRNA vaccine (BNT162b2 or mRNA-1273) and a single dose of adenovirus-vector vaccine (Ad26.COV2.S) were considered as fully vaccinated. For patients who experienced disease progression during the time of sample collection, the diagnosis at the time of 1st dose of vaccination was adopted. For SMM patients with treatment history (summarized in Table S1C), the regimen applied closest to the date of 1st dose of vaccine was adopted as the regimen for each patient. SMM patients with the treatment withdrawal date more than 2 years ago were considered "untreated". All specimens were de-identified prior to processing and antibody testing for all plasma specimens was performed in a blinded manner.

ELISA assay to detect IgG antibodies against SARS-CoV-2

ELISA assay to detect anti-SARS-CoV-2 antibodies was performed as previously described with some modifications (Yue et al., 2020). In brief, 384-well ELISA plates (ThermoFisher #464718) were coated with 50 µl/well of 500 ng/ml SARS-CoV-2 S protein in coating buffer (1 capsule of carbonate-bicarbonate buffer (Sigma #C3041100CAP) per 100 mL Milli-Q H_2O) for 30 minutes at room temperature. Plates were then washed 3 times with 100 µl/well of wash buffer (0.05% Tween-20, 400 mM NaCl, 50 mM Tris pH 8.0 in Milli-Q H_2O) using a Tecan automated plate washer, followed by blocking using 100 µl/well of blocking buffer (1% BSA,

140 mM NaCl, 50 mM Tris pH 8.0 in Milli-Q H_2O) for 30 minutes at room temperature. After washing 3 times as described above, 50 µl of diluted plasma sample (in dilution buffer; 1% BSA, 0.05% Tween-20, 140 mM NaCl, 50 mM Tris (pH 8.0) in Milli-Q H_2O) were added to each well and incubated for 30 minutes at 37°C. After washing 5 times, 50 µl of diluted detection antibody solution (HRP anti-human IgG; Bethyl Laboratory #A80-104P) was added to each well and incubated for 30 minutes at room temperature. Following an additional 5 washes, 40 µl of TMB peroxidase substrate (Thermo Fisher #34029) was added to each well and incubated at room temperature for 3 minutes. Then, the reaction was stopped by adding 40 µl of stop solution (1 M H_2SO_4 in Milli-Q H_2O) to each well. OD was read at 450 nm and 570 nm on a PHERAstar FSX plate reader. The final data used in the analysis was calculated by subtracting 570 nm background from 450 nm signal.

TR-FRET assay to detect IgG antibodies against SARS-CoV-2

TR-FRET assay to detect anti-SARS-CoV-2 antibodies was performed as previously described (Yue et al., 2020). As a positive control, we used recombinantly expressed SARS-CoV-1 IgG antibody CR3022 which has been shown to cross-react with spike protein of SARS-CoV-2 (ter Meulen et al., 2006; Tian et al., 2020). Plasmids encoding CR3022 was a gift from Galit Alter, MGH, Boston, MA. Titration of CR3022 IgG antibody or dilution of tested human plasma samples was added to assay mix with final concentrations of 7.5 nM Terbium-labeled SARS-CoV-2 Spike protein, 250 nM BODIPY-labeled αIgG in a buffer containing PBS, 0.05% Tween-20 (Sigma Aldrich #P9416). Plasma samples were diluted in the buffer containing 50 mM Tris pH 8.0, 140 mM NaCl, 0.05% Tween-20, and 1% BSA (Cell Signaling Technology #9998S). TR-FRET assays were performed in a 384-well microplate (Corning, #4514) with 15 µL final assay volume. Before TR-FRET measurements were conducted, the reactions were incubated for 1 h at room temperature. After excitation of terbium fluorescence at 337 nm, emission at 490 nm (terbium) and 520 nm (BODIPY) were recorded with a 70 µs delay over 130 µs to reduce background fluorescence and the reaction was followed by >20 or > 100-second cycles of each data point using a PHERAstar FS microplate reader (BMG Labtech). The TR-FRET signal of each data point was extracted by calculating the 520/490 nm ratio.

TR-FRET assay to examine the immunoglobulin interference

To examine the immunoglobulin interference in TR-FRET assay, we prepared two groups of samples; samples from individuals without plasma cell dyscrasias (n = 3; provided from the

Fischer Lab) and samples from SMM patients (n = 4) with high serum IgG titers (**Figure S1B**). We added CR3022 IgG, to these samples with several concentration (range from 1 x 10^{-2} mg/dL to 1 x 10^{-8} mg/dL). If there is any interference due to serum IgG, the TR-FRET measurements using patient's samples were expected to come back negative or unexpectedly low values compared to control samples.

Statistical analysis

Continuous variables are presented as medians with the range. Categorical variables are shown as a percent and number of subjects. We applied multiple linear regression in our multivariate analyses. We reported β coefficient, 95% CIs, and p-values, where applicable. The Wilcoxon matched-pairs signed rank test or Mann-Whitney test were used to compare groups. A two-sided alpha <0.05 was considered statistically significant. All statistical analysis was done using either Graph Pad Prism (version 9.0.1) or R software (version 4.1.1).

Supplemental References

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